Bnr formin linking actin in *Ashbya gossypii*


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Introduction

• The SPB is the fungal analogue to the centrosome of higher cells and consists of several proteinaceous layers.

• Sporulation-specific components are recruited to the SPB to form the meiotic outer plaque (MP).

• Membrane-coated vesicles are acquired by the MP, where they fuse to form the pro- or forespore membrane precursor.
Introduction

• *A. gossypii* grows filamentous with multiple haploid nuclei in a single cytoplasm.

• AgBnr2 is a protein of the formin family, which is primarily known for its role in the regulation of fungal cell shape and is an important player in sporulation. It also links actin to the SPB during this process.
Procedures

• PCR-Based gene targeting or transforming with Plasmids

• Cell Staining for microscopy

• Extraction of Nuclei by differential centrifugation.
Question

• How vesicles are targeted to the MP and how the shapes of the spores might be controlled, especially for fungal spores that are morphologically more complex than the round spores of *S. pombe* and *S. cerevisiae*?
DIC AgAbp140-GFPAgSpc42-DsRED overlay
Other Findings

• The FH1/FH2 region of AgBnr2 increases filamentous actin in vivo

• AgBnr2 colocalizes with actin filaments during sporulation
Conclusions

• AgBnr2 action at the SPB seems to occur only during sporulation. Recent work on *S. cerevisiae* sporulation showed that several components that are involved in polar growth control and cytokinesis also are involved in spore formation.

• efforts to characterize the interactions of AgBnr2 with components of the SPB resulted in various weak two-hybrid interactions with several components
• results indicate that actin and AgBnr proteins might play an important role in this process.

• Future Question- whether AgBnr2 has functions at the SPB other than regulating actin during sporulation.

• during sporulation, AgBnr2 localizes to the SPB by interacting with components of the g-tubulin complex, the outer plaque and the inner layer. However, the exact timing, stoichiometry and role of AgBnr2 within the SPB structure are not clear.
• Work provides evidence that Bnr-like proteins play a role at the SPB during sporulation and suggests a novel role for formin proteins in linking actin polymerization to the SPB.

• *Ashbya gossypii*, localizes to the spindle pole body (SPB), the fungal analogue of the centrosome of metazoans. This protein plays an important role in the development of the typical needle shaped spores of *A. gossypii*, as suggested by several findings.
Summary

• First, down regulation of AgBNR2 causes defects in sporangium formation and a decrease in the total spore number.
• Second, a fusion of AgBNR2 to GFP that is driven by the native AgBNR2 promoter is only visible in sporangia.
• Third, AgBnr2 interacts with a AgSpo21, a sporulation specific component of the SPB.
• There’s evidence that AgBnr2 might nucleate actin cables, which are connected to SPBs during sporulation.